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Natural History of Colorectal Carcinoma: Can the Tumor Volume Doubling Time Be Predicted by Radiologic Findings or Immunohistochemical Variables?

IKUKO IWASHITA, MD,[1]* TOSHIHIKO UEYAMA, MD,[1] AKINORI IWASHITA, MD,[3] KENJI KAWAMOTO, MD,[1] SHINJI KITAGAWA, MD,[2] MAKOTO MOTOOKA, MD,[1] TAKASHI UTSUNOMIYA, MD,[4] AND KOUJI MASUDA, MD[1]

[1]*Department of Radiology, Faculty of Medicine, Kyushu University, Fukuoka, Japan*
[2]*Faculty of Medicine, Fukuoka Univeristy, Fukuoka, Japan*
[3]*Department of Pathology, Fukuoka University Chikushi Hospital, Fukuoka, Japan*
[4]*Faculty of Medicine, Kyushu University, Fukuoka, Japan*

Background and Objectives: The factors influencing the growth rate of colorectal carcinoma have not been determined. The aim of this study was to clarify the relationship between the doubling time (DT), morphology, and proliferating cell nuclear antigen, Ki-67 and p53 immunohistochemistry in colorectal carcinoma.

Methods: Thirty-three patients (37 lesions) were studied retrospectively. The DT was calculated and correlated with the initial and final tumor size, morphologic shape, and immunohistochemical results.

Results: The DT ranged from 2.4 to 48.0 months (mean: 12.0 months). The mean DT of the early-stage carcinomas was significantly longer than that of the advanced carcinomas. In the latter group, both slowly growing and rapidly growing tumors were observed. The DT showed no correlation with the initial or final size and shape of the tumors on radiographs, or with the immunohistochemical results.

Conclusions: Our data revealed that it is not possible to evaluate the growth rate of colorectal carcinomas based on their morphological shape, cellular proliferative activity, or tumor suppressor gene activity.

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KEY WORDS: **growth rate; cellular proliferative activity; tumor suppressor gene; colon carcinoma**

INTRODUCTION

The natural history of colorectal carcinoma has been a primary concern of many investigators since the 1950s. There have been two main approaches to this problem. The first approach is to evaluate retrospectively tumor growth rates using serial imaging studies. Since the introduction of the tumor volume doubling time (DT) by Collins et al. [1,2] as an indicator of the growth rate, many reports concerning the DTs of human neoplasms have been published. However, there have been few reports regarding primary colorectal carcinomas, because of the difficulty in obtaining follow-up imaging studies. Welin et al. [3], reporting the growth rate of different morphologic types of primary colorectal carcinoma, found that DTs ranged from 138 to 1,155 days. They also showed that many colorectal carcinomas grow slowly. On the other hand, rapidly growing cases have been reported by other authors [4,5]. It is well known that colorectal carcinomas are divided into two groups, i.e., slowly and rapidly growing carcinomas, according to the DT. Whereas the DTs of colorectal carcinomas have

*Correspondence to: Ikuko Iwashita, MD, Department of Radiology, Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan. Fax No.: (81)92-642-5708.

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been elucidated as mentioned above, they are not generally available for clinical purposes, because once a carcinoma is diagnosed or suspected, it is usually resected.

Because the calculation of the DT is difficult in colorectal carcinoma, interest has arisen in whether or not factors available at the time of diagnosis can predict the growth behavior of the tumor. This is the second approach of evaluating tumor growth. Histopathologic findings that may reflect the clinical growth rate of colorectal carcinomas have been evaluated [6–11]. The prognostic value of some histopathologic parameters, such as lymphatic infiltration [6], tubular configuration [6], the pattern of growth [6], the degree of differentiation [8], eosinophil and mast cell infiltration [9], tumor morphology [10], and venous invasion [11], has been confirmed previously. However, the potentially subjective nature of these histopathologic observations has prompted investigators to identify more objective prognostic parameters.

With the development of immunohistochemical analysis it is now possible to assess proliferative activity and tumor suppressor gene activity in formalin-fixed, paraffin-embedded human tissues. Many reports have been published concerning proliferative activity and tumor suppressor gene status in colorectal carcinomas [12–20]. However, the usefulness of proliferative activity and tumor suppressor gene status in colorectal carcinomas has never been confirmed. The DT is an accepted indicator of the tumor growth rate in human solid tumors [1–5,21]. Only one comparative study between the DT and tumor proliferative activity has been performed in lung adenocarcinoma, by Ogura et al. [21] using nucleolar organizer regions visualized by silver staining (AgNOR). In their report, an inverse correlation was shown between the DT and the mean number of AgNORs. However, no comparative study between the DT and other immunohistochemical data has been done on colorectal carcinoma. It is not known whether proliferative activity is useful for evaluating the clinical growth rate of colorectal carcinoma.

The aim of this study was to clarify the natural history of colorectal carcinoma and to evaluate the relationship between the DT, radiologic findings, and the immunohistochemical parameters, proliferating cell nuclear antigen (PCNA), Ki-67, and p53.

MATERIALS AND METHODS

Study Population

A total of 50,137 double-contrast barium enema (BE) studies were performed in 12 institutions or hospitals during the 18-year period ending in October 1993. Medical records were reviewed, and patients with an established diagnosis of colorectal carcinoma who underwent BE studies immediately (less than 2 weeks) before and at least 10 months prior to resection of colorectal carcinoma were identified. The films of the initial BEs were reviewed by radiologists at each institution or hospital to identify colorectal carcinomas that were overlooked at the initial interpretation. In comparing these films with the final films of BEs performed immediately prior to the resection, only the lesions that were located in exactly the same region in all projections were selected.

A total of 37 primary colorectal carcinomas were thus collected in 33 patients. They were 21 men and 12 women, with ages ranging from 54 to 84 years (mean: 66 years). BE examinations were performed from 2 to 11 times (2 times in 27 patients, 3 times in 4 patients, 4 times in 1 patient, and 11 times in 1 patient). Thirty-two tumors were demonstrated on 2 BEs; the other tumors were demonstrated on more than 2 BEs. The follow-up period ranged from 10 to 129 months (mean: 36 months). The diagnosis was established by surgical resection in 27 patients and by endoscopic resection in 4 patients. Endoscopic biopsy alone was performed in 2 patients: 1 patient with an advanced carcinoma of the rectum was considered inoperable because of the aggressiveness of the lesion and 1 patient died of other causes. Thirty of 33 (90.9%) patients had a solitary carcinoma. The remaining 3 of 33 (9.1%) had multiple carcinomas (2 in 2 patients and 3 in 1 patient).

Histopathologic findings were assessed by an experienced gastrointestinal pathologist (A.I.), according to the criteria of the Japanese Research Society for Cancer of the Colon and Rectum [22]. Among the 37 lesions, 2 were obtained by endoscopic biopsy only, so the depth of tumor invasion, venous invasion, lymphatic invasion, and lymph node metastasis were assessed in only 35 lesions.

Radiologic Evaluation

Interval changes in the size and morphology of the carcinomas during the follow-up period were again assessed by 3 gastrointestinal radiologists (I.I., T.U., K.K.) without knowledge of the histopathologic results. The feasibility of measuring changes in size by serial measurement of primary colonic tumors outlined by BE examinations has been reported previously by Spratt and Ackerman [23]. On the basis of their study, the largest diameter of the tumor was measured on the initial and follow-up double-contrast radiographs on roughly equivalent projections. When calculating the size of a tumor, correction was made for differences in magnification, as judged by the size of the vertebrae or pelvic bones. The DT (Doubling Time) was calculated according to the equations of Collins et al. [1,2] and Kusama et al. [24] as follows: $DT = t/10 (\log a2 - \log a1)$, where t = the interval between measurements (in months), $a1$ = the tumor diameter at the initial examination, and $a2$ = the tumor diameter at the final examination. For the evaluation of the initial shape of tumors, tumors were divided into 2 groups: early and advanced carcinoma.

Because the histopathologic studies were not available at the time of the initial BE studies, this radiologic classification of the tumors was based purely on the findings of the initial BE studies. The presence of a consistent deformity of the colonic haustration at the site of the tumor was used as an indicator of advanced carcinoma. The morphology of the early carcinomas was classified as follows: type 1-1, superficial elevated lesion with a smooth, granular, or nodular surface; type 1-2, superficial elevated lesion with a central depression; type 2, sessile lesion with a smooth surface; and type 3, pedunculated lesion. The morphology of the advanced carcinomas was classified according to Borrmann's [25] classification (types B-1, B-2, B-3, B-4, and B-5). To evaluate the final shape of tumors, tumors were classified according to Borrmann's classification based on the histopathologic findings.

Immunohistochemical Staining

For p53, PCNA, and Ki-67 immunostaining, paraffin-embedded sections, which were obtained from approximately the same portion of the tumors as the histopathologic sections, were placed on poly-L-lysine-coated glass slides and air-dried at room temperature. Deparaffinized and dehydrated sections were heated in a microwave oven (Hitachi, MR-M220, Tokyo, Japan) for seven 3-min cycles in citrate buffer to retrieve antigenic activity and cooled for 60 min at room temperature. Endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxidase in methanol for 20 min at room temperature. After blocking non-specific reactions with 10% normal rabbit serum, the sections were first incubated with p53 antibody (Novocastra Laboratories, Ltd., Newcastle, UK) for 1 hr at a dilution of 1:100, PCNA antibody (Dako, Glostrup, Denmark) for 1 hr at a dilution of 1:100, or Ki-67 antibody (Immunotech SA, Marseille, France) for 1 hr at a dilution of 1:100. The sections were then incubated with biotinylated anti-mouse IgG horse BA-2000 for 30 min at a dilution of 1:200 and alkaline phosphatase-labeled streptavidin (Dako) for 30 min at a dilution of 1:100. Careful rinses were done with several changes of phosphate-buffered saline (PBS) between each stage of the procedure. The color was developed with alkaline phosphatase. The sections were counterstained with hematoxylin and mounted.

To ensure consistency of immunohistochemical staining between batches, a known positive control was included in each round: tonsil for Ki-67 and known positive colonic carcinomas for PCNA and for p53. Negative controls were included by performing duplicate assays, on one of which the primary antibody was replaced by PBS.

For semiquantitative analysis, a grading system was devised in which the whole section was assessed at a low power (×100) in an effort to take better account of tumor heterogeneity. Carcinomas were allocated to grades 1–4 [labeling grade (LG)] if the observer estimated tumor cell nuclei to be positive in 0–25%, 26–50%, 51–75%, or 76–100% of the tumor cells, respectively (Fig. 1A–D). The LG was evaluated before the quantitative analysis in all carcinomas.

For quantitative analysis, the sections were counted at a high power (×400). Although some cells stained more strongly than others, it is well known that the condition of fixation and paraffin embedding influence the immunoreactivity, so all identifiable staining was regarded as positive. Nuclei from 1,000 tumor cells were counted in areas of the tissue sections that showed a predominant pattern of a high frequency (HF) of positive nuclei (HF area). The labeling rate (LR) was calculated in the HF area by the following equation: LR = (No. of positive nuclei in HF area/total nuclei) × 1,000. On initial review of the stained sections of the carcinomas, it was apparent that while sections tended to show a predominant pattern of a HF of positive nuclei, some areas of similar histology showed a lower frequency of positive nuclei. The labeling score (LS) was derived to take account of this variation in the frequency of positive nuclei, according to the method of Johnston et al. [12]. The LS is defined as the fraction of positive nuclei among 1,000 nuclei in a HF area adjusted by the fraction of the HF area in the whole section. Therefore, the LS is calculated by the following equation: LS = LR × HF area/total area. The ratio between the HF area and the total area represents the heterogeneity of the carcinoma.

Statistical Analysis

The results of the radiologic and immunohistochemical studies were correlated with the DT. Then, the mean values of DT, LR, and LS were compared between or among the groups by either the Wilcoxon rank-sum test or the Kruskal-Wallis test. In this calculation, tumor size was categorized into 3 levels according to the approximate tertile. The relationship between the DT and the LR or LS was examined with the Spearman correlation coefficient using the Statistical Analysis System (SAS; SAS Institute, 1985). *P*-values were based on 2-tailed test, and $P < 0.05$ was considered significant.

RESULTS

Clinicopathologic Findings

There were 27 well-differentiated adenocarcinomas, 9 moderately differentiated adenocarcinomas, and 1 poorly differentiated adenocarcinoma (Table I) according to the predominant differentiation of the tumors. Twenty-six tumors were at an advanced stage at the time of resection, 9 tumors were at an early stage, and the remaining 2 had an unknown depth of invasion.

Marked extraluminal growth was not observed. There

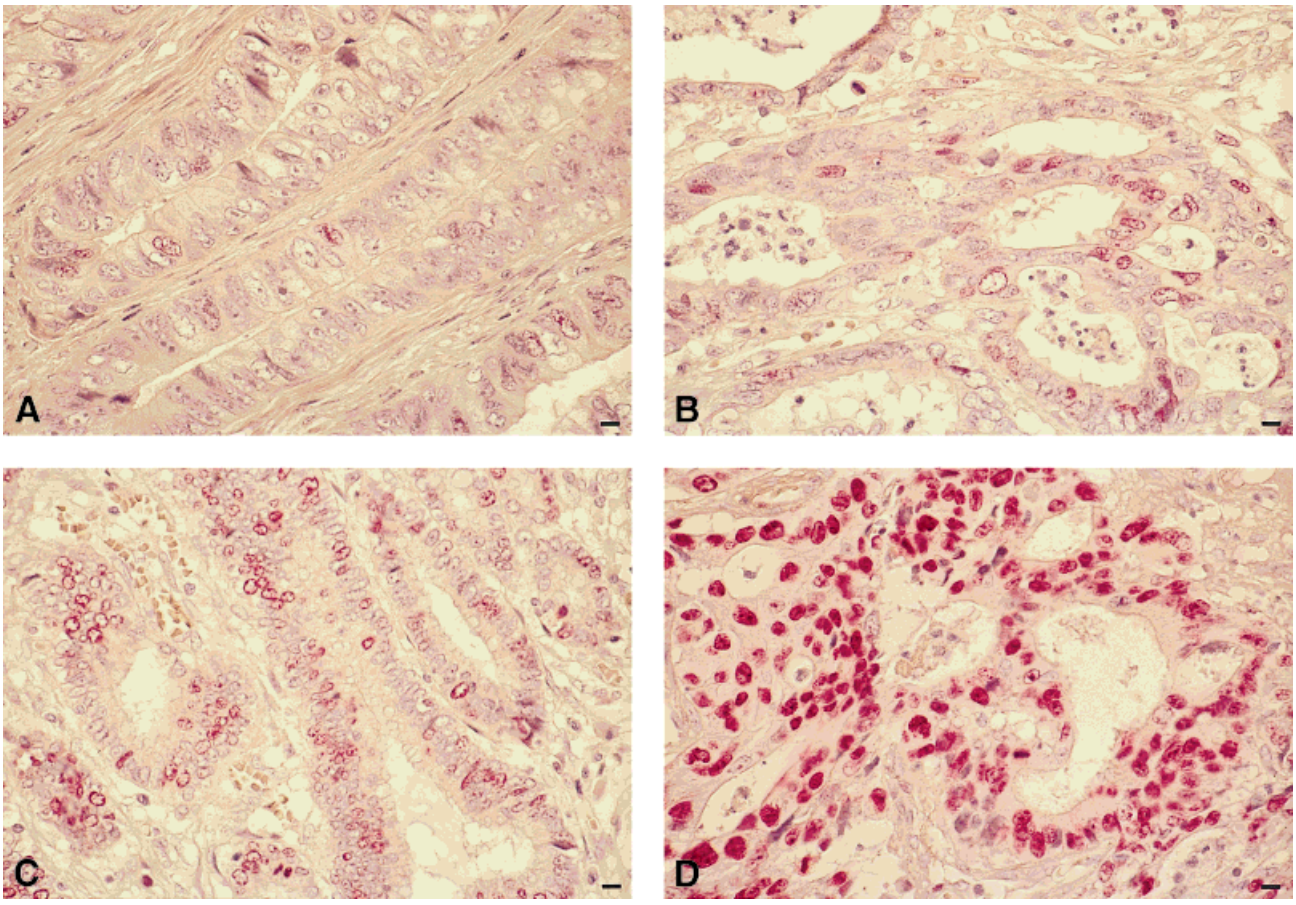

Fig. 1. Grading of immunohistochemical staining of colorectal carcinomas. Cells stained in red were assessed as positive. **A:** Grade 1. Positive in 0–25% of tumor cell nuclei. **B:** Grade 2. Positive in 26–50% of tumor cell nuclei. **C:** Grade 3. Positive in 51–75% of tumor cell nuclei. **D:** Grade 4. Positive in 76–100% of tumor cell nuclei. Bars = 10 μm.

were no tumors with disproportionate submucosal growth compared with the growth on the luminal surface.

Radiologic Findings and DT

The diameters of the tumors measured on the initial BE ranged from 6 to 45 mm (mean: 19 ± 11 mm). On final examination, the diameters of the carcinomas ranged from 12 to 140 mm (mean: 46 ± 25 mm). Twenty-eight carcinomas were considered to be at an advanced stage on the final examination. The remaining 9 were early-stage lesions. These findings were the same as the histopathologic findings, except for the 2 unresected tumors. In the 28 advanced carcinomas, the initial tumor size was 6–45 mm and the final tumor size was 19–140 mm. In the 9 early carcinomas, the initial tumor size ranged from 8 to 40 mm and the final tumor size from 12 to 70 mm.

During the follow-up period, 36 tumors increased in diameter; the remaining 1 tumor showed no change in size. The DT therefore was calculated in 36 lesions. The diameter was plotted against the observation time on a semilogarithmic scale for early carcinomas (Fig. 2) and advanced carcinomas in which there were 2 observations

TABLE I. Histopathologic Findings in 37 Colorectal Carcinomas

	No. of tumors
Differentiation	
Well	27
Moderate	9
Poor	1
Depth of invasion	
Mucosal invasion	5
Submucosal invasion	4
Muscularis propria invasion	6
Subserosal invasion	20
Unknown	2
Venous invasion	
None	12
Minimal	22
Moderate to massive	1
Unknown	2
Lymphatic invasion	
None	10
Minimal	22
Moderate to massive	3
Unknown	2
Lymph node metastasis	
Negative	22
Positive	7
Unknown	8

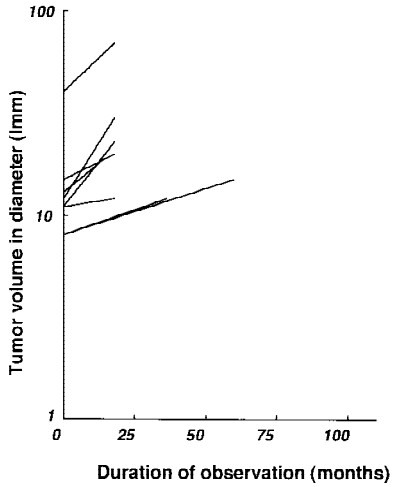

Fig. 2. Relationship between the observation period and the tumor size in 9 early carcinomas. lmm = loge mm.

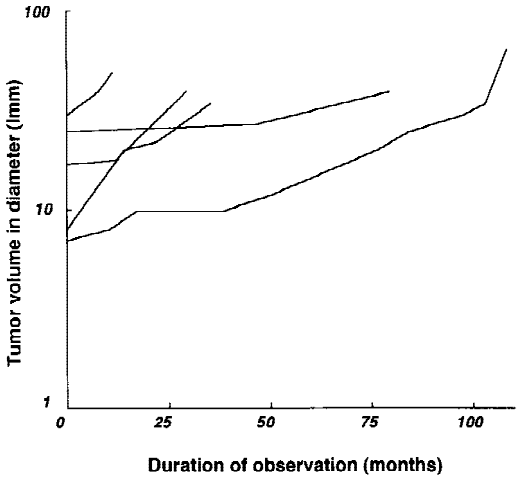

Fig. 4. Relationship between the observation period and the tumor size in 5 advanced carcinomas in which there were at least 3 observations. lmm = loge mm.

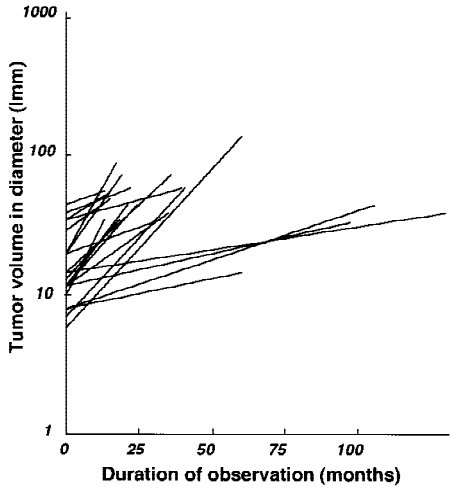

Fig. 3. Relationship between the observation period and the tumor size in 23 advanced carcinomas in which there were 2 observations. lmm = loge mm.

(Fig. 3). The DT ranged from 2.4 to 48.0 months (mean: 12.0 ± 12.0 months). The mean DT of the early carcinoma group (20.6 ± 17.0 months) was significantly longer than that of the advanced carcinoma group (9.7 ± 9.0 months) ($P = 0.027$). There appeared to be 2 types in the advanced carcinoma group: slowly and rapidly growing tumors (Fig. 3). All advanced carcinomas with more than 2 follow-up BEs (5 cases) showed an accelerated growth pattern in the late stages (Fig. 4).

Thirty-one tumors were considered to be at an early stage on the initial examination. The initial tumor shapes were classified as follows: type 1-1 in 6, type 1-2 in 4, type 2 in 18, and type 3 in 3. The remaining 6 were initially advanced carcinomas. There was no statistically significant correlation between the initial shape of the tumors and the DT ($P = 0.069$). Eight tumors had a central depression at the initial examination. The mean DT of the lesions with a central depression was not statistically different from the lesions without central depression ($P = 0.076$). Three tumors had a stalk at the initial examination. The mean DT of the pedunculated lesions was not significantly different from that of lesions without a stalk ($P = 0.095$). Seven tumors had a lobular surface. The remaining 30 had a smooth surface. The mean DT of the lobulated lesions was not statistically different from that of lesions with a smooth surface ($P = 0.874$). At the final examination, 28 lesions were considered to be at an advanced stage: B-1 in 2, B-2 in 23, and B-3 in 3. The remaining 9 were considered to be at an early stage. There was no statistically significant correlation between the final shape of the tumors and the DT ($P = 0.368$).

A case example is presented. A 73-year-old man underwent a BE because of lower abdominal pain. A superficial elevated lesion with a central depression (type 1-2) measuring 7 mm in diameter was noted in the transverse colon (Fig. 5A). Endoscopic examination failed to detect the lesion, and no further workup was pursued at that time. After 16 months, a follow-up BE was performed, and a superficial elevated lesion with a central depression measuring 19 mm in diameter was seen at the same location (Fig. 5B). On repeated colonoscopy the lesion was detected and the tumor was resected endoscopically. The tumor was a well-differentiated adenocarcinoma involving the muscularis propria with lymphatic and venous invasion. Because of the coexistence of a small polypoid lesion, the location of the tumor could be confirmed.

Immunohistochemical Findings and DT

Semiquantitative grading system. There was no statistically significant correlation between the DT and the LG of the PCNA, Ki-67, or p53 (Table II).

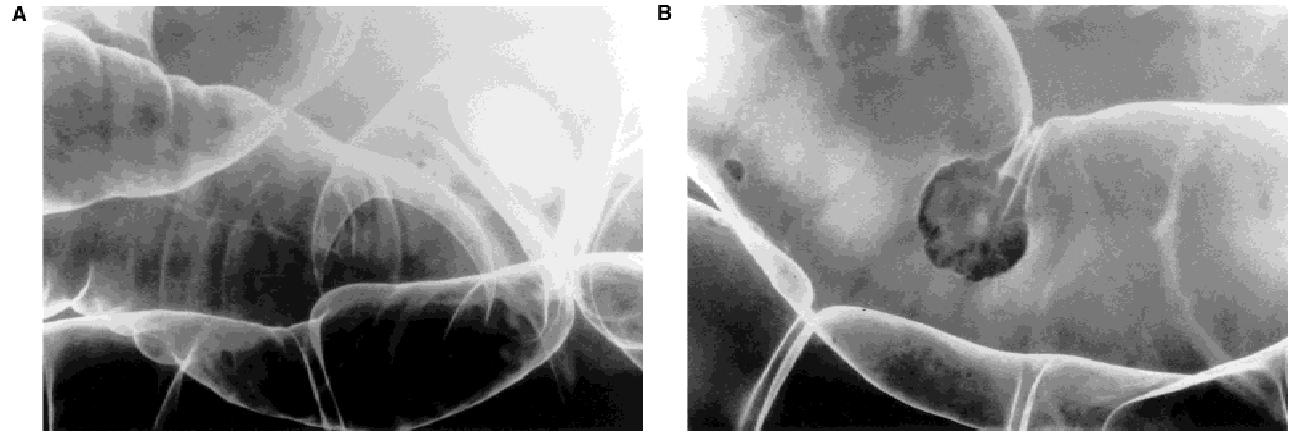

Fig. 5. Case presentation: 73-year-old man. **A:** X-ray obtained on 17 August, 1991. A superficial, elevated lesion 7 mm in diameter with a central shallow depression is demonstrated. **B:** Sixteen months later, the lesion increased to 19 mm in diameter. The calculated DT of this lesion is 3.7 months.

TABLE II. Correlation Between Immunohistochemical Staining and DT in 36 Colorectal Carcinomas*

	Mean DT ± SD (months)		
G	Ki-67	PCNA	p53
1	10.6 ± 11.7	10.5 ± 8.8	12.4 ± 13.9
2	13.5 ± 15.2	13.4 ± 19.4	9.7 ± 6.7
3	12.8 ± 12.9	9.1 ± 7.9	12.2 ± 8.4
4	10.5 ± 7.9	13.2 ± 12.2	12.9 ± 13.9

**P*-values by the Kruskal-Wallis test were 0.998 for Ki-67, 0.722 for PCNA, and 0.929 for p53. Corresponding *P*-values for the Spearman correlation coefficient were 0.004, 0.061, and 0.015, respectively.

Quantitative Analysis

PCNA. The LR of the PCNA varied from 208 to 953 (mean: 839 ± 139). The LS of the PCNA varied from 117 to 873 (mean: 540 ± 211). There was notable intratumoral heterogeneity in staining in many cases. The LR and the LS of the PCNA were independent of tumor size and radiographic appearance and had no significant correlation with the DT (Fig. 6A,B).

Ki-67. The LR of the Ki-67 antigen varied from 538 to 970 (mean: 773 ± 126). The LS varied from 34 to 970 (mean: 361 ± 254). Staining heterogeneity was slightly more prominent than with the PCNA. The Ki-67 LR and LS did not correlate with the radiographic appearance of the tumor nor with the DT (Fig. 7A,B).

DISCUSSION

Collins et al. [1,2] claimed that if the growth rate of a hypothetical tumor was constant, it could be described in terms of the DT, as the half-life describes the decay of a radioactive isotope. They showed that the growth rate of pulmonary metastases in patients with primary colorectal carcinomas is of considerable importance in understanding the growth rate of primary lesions. They concluded that the growth rate of pulmonary metastases was quite slow in most instances, and assumed that primary colorectal carcinomas also grew slowly. Subsequently, the DT has been used to represent the growth rate of a variety of human neoplasms, including colorectal carcinomas [1,3–5,26], metastatic tumors [1,2], and lung adenocarcinomas [21]. Shackney et al. [27] have collected data on the DT of human solid tumors from the literature and have calculated the mean DT of various tumors (testicular carcinoma, breast carcinoma, osteogenic sarcoma, Ewing sarcoma, Hodgkin disease, non-Hodgkin lymphoma, epidermoid carcinoma, adenocarcinoma of the lung, adenocarcinoma of the colon, and fibrosarcoma). They divided these tumors into 3 groups according to the DT and showed that the mean DT of colorectal adenocarcinoma exceeded 70 days and thus belonged to the most slowly growing group. Subsequently, for primary colorectal carcinomas similar DTs have been reported. Figiel et al. [26] analyzed sequential single-contrast BEs and presented examples of slowly growing colorectal carcinomas. Welin et al. [3] investigated serial double-contrast BEs in 20 patients with primary colorectal carcinoma and obtained DTs ranging from 138 to 1,155 days. The results of most previous studies support the slow growth rate of colorectal carcinoma. However, other investigators have reported the existence of rapidly growing colorectal carcinomas [4,5]. The results of these studies demonstrate the existence of both slowly and rapidly growing colorectal carcinomas, the former being far more frequent. Our results showed that the DT of colorectal carcinomas ranged widely, in agreement with previous reports.

A correlation between the DT and the morphologic shape was sought in our study. No significant correlation was found between the DT and the initial or final tumor shape. Ushio and Ishikawa [28] correlated the details of the initial shape of colorectal carcinomas on double-contrast BE with the DT and stated that colorectal car-

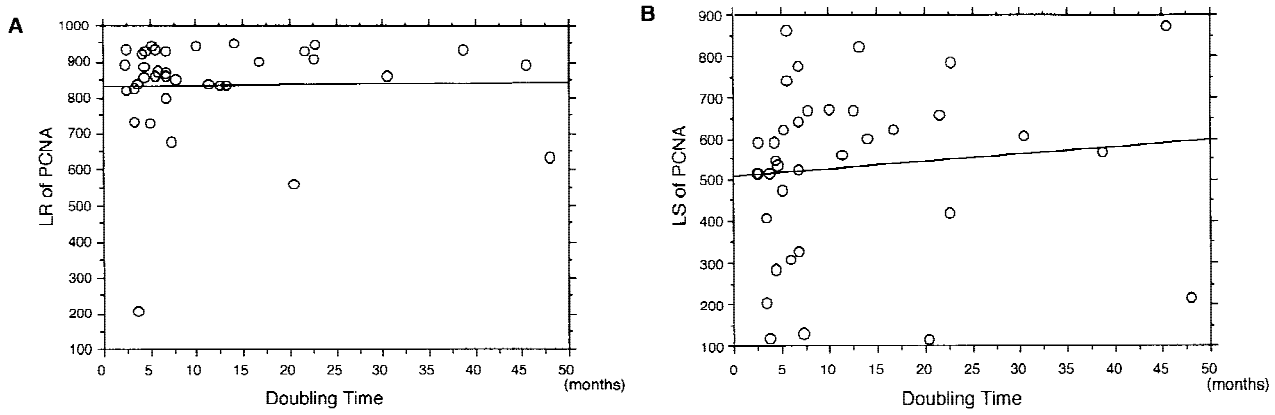

Fig. 6. Correlation between the LR **(A)** and LS **(B)** of PCNA and the DT.

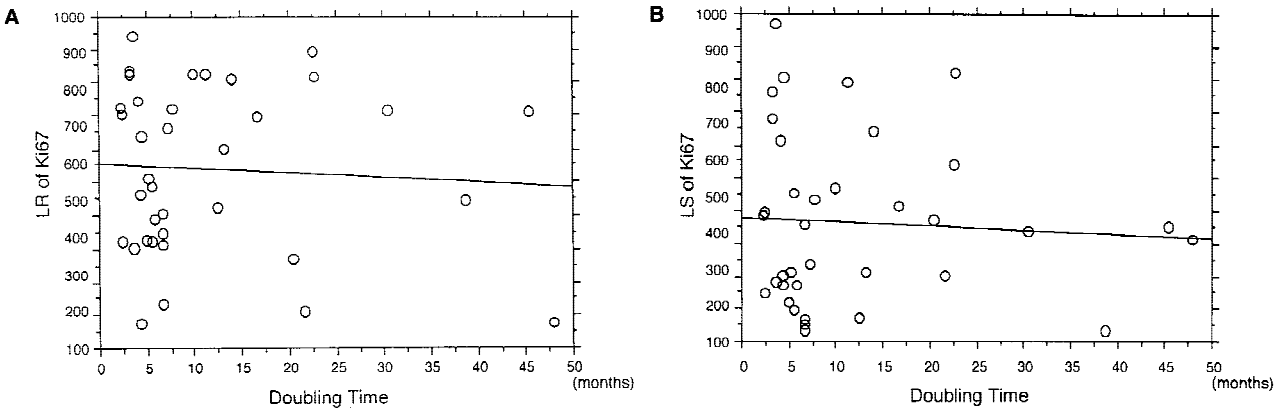

Fig. 7. Correlation between the LR **(A)** and LS **(B)** of Ki-67 and the DT.

cinomas with central depressions have short DTs. Welin et al. [3] reported a case of pedunculated polyp which was in an equilibrium status for 7 years. They suspected that a pedunculated morphologic configuration would increase the likelihood of an equilibrium between the rate of cellular loss by desquamation and the rate of cellular gain from neoplastic cellular proliferation. However, they concluded that there was an overlap of growth rates for different morphologic types of tumors. In our data, the DTs of the tumors with distinct central depressions were not significantly shorter than those reported in the literature. Our study showed no statistically significant difference in DT between pedunculated tumors and sessile tumors. This might reflect the overlap of growth rates for different morphologic types of tumors in agreement with Welin et al. [3].

The development of monoclonal antibodies has opened new frontiers in investigating cellular proliferation. Many studies have assessed the clinical implications of the proliferative activity of human colorectal carcinomas based on a comparison with their histologic findings [12–20]. Tumor cell proliferative activity surveys can ideally provide a useful addition to the understanding of a tumor's biologic aggressiveness.

Phosphoprotein p53 has attracted attention as a cancer suppressor gene [29,30]. Recently, p53 has been implicated in the final step of carcinogenesis [31]. p53 is located on the short arm of chromosome 17 and is mutated in various types of carcinomas [32–35]. We expected a high level of p53 overexpression in our study because the lesions we examined were all carcinomas. However, the LG of p53 varied over a wide range and did not correlate with the DT.

The PCNA, recognized to be associated with cell division, is defined by its reaction with an antibody found in systemic lupus erythematosis by Miyachi et al. [36]. The monoclonal antibody to PCNA, PC10, provides a new approach for determining proliferative activity in paraffin-embedded tissue. There is much evidence that the relationship between PCNA expression and prognosis varies in different types of tumors [37–40]. The reported percentage of PCNA positive cells in colorectal carcinomas ranges from 20.0 to 69.0% [16,19]. In our study, the percentage of PCNA positive cells in the HF area (LR) varied from 20.8 to 95.3% (mean: 83.9%) and the percentage adjusted by the factor of the heterogeneity (LS) varied from 11.7 to 87.3% (mean: 54.0%). These data are not different from those reported by others. In

our data, the difference between LR and LS was about 20%. In the majority of our cases, the carcinomas were very large in size at the time of resection, and an intratumoral heterogeneity of stains was clearly seen. This seems to offer an explanation for the difference between LR and LS. Jain et al. [40] used a PCNA index (percentage of positive cells/1,000 tumor cells) and a semiquantitative PCNA grading system (based on estimates of less than or more than 50% positive tumor cells in the whole section), and showed that the ability of semiquantitative PCNA grading to allow for intratumoral variation may have advantages over absolute counting, which is prone to sampling error when tumor heterogeneity is a major factor. We used the LG for semiquantitative analysis, in which the tumors were allocated to grades 1–4. The LS correlated with the LG in our study. The LS we used is a quantitative analysis, so it may be more useful for assessing a tumor's mean proliferative activity. Additionally, it corrects for intratumoral heterogeneity, so it may be more useful than the random counting used by previous authors. However, the question whether the proliferative activity of one tumor can be best represented by mean activity or by highest activity is not answered. So evaluation of both LR and LS may be necessary.

The Ki-67 monoclonal antibody recognizes a nuclear antigen exposed only in proliferating cells during all phases of the cycle except G0 [41]. The suitability of monoclonal antibody Ki-67 for the detection of the growth fraction in tumors has previously been reported in studies of malignant lymphomas [42], breast carcinomas [43], gastric carcinomas [44], and colon carcinomas [17,18]. Suzuki et al. [18] reported that the Ki-67 labeling index (the number of Ki-67 positive cells divided by the total number of tumor cells) for colorectal carcinoma ranged from 15.7 to 63.6% (mean: 38.5%). They also reported that the proliferative activity of cancer tissue is higher than the activity of normal tissue, although the proliferative activity of cancer tissue has a wide range. In our study, the percentage of Ki-67 positive cells in HF areas ranged from 53.8 to 97.0% (mean: 77.3%). This mean value is higher than in previous reports [16–19,42]. This is probably because in previous studies the positive cells were counted in randomly selected fields, so the counts represented the mean proliferative activity, whereas in our study the positive cells were counted in HF areas, so the data represented the highest activity. We assessed the proliferative activity by LS, which corrected for intratumoral heterogeneity. The LS of Ki-67 ranged from 34.0 to 97.0% (mean: 36.1%), in line with previous reports [17,18]. Porschen et al. [17] reported that marked heterogeneity of Ki-67 expression existed in different areas of the same tumor. They suggested that the Ki-67 index is nearly completely independent from clinicopathologic variables and that the observed heterogeneity of the Ki-67 expression within the same tumor may reflect differences in growth kinetics. The Ki-67 index may therefore be of prognostic value. In our study, intratumoral heterogeneity of expression was slightly less marked with PCNA than with Ki-67. This is in agreement with the results of Rosa et al. [45] in gastric carcinomas. However, Berenzi et al. [20] have reported that no relevant variation was seen in Ki-67 expression between central and peripheral sections of tumors. Based on the assumption that the tumor growth is due to the mean proliferative activity, the LS may be useful for assessing the tumor growth. On the other hand, based on the assumption that the tumor growth is due to the highest proliferative activity in the tumor, the LR may be more useful. Since it is not known which assumption is correct, to use both counting methods (LR and LS) is important.

A comparative analysis of Ki-67 and PCNA expression in gastric carcinoma has been performed by Rosa et al. [45]. They found no correlation between PCNA and Ki-67 expression, and have suggested that both inter- and intratumoral heterogeneity in proliferative activity are reflected in a high standard deviation. Our results agree with their report. Therefore, although PCNA and Ki-67 represent tumor proliferative activity, the two methods might not represent the same process in tumor proliferation.

The usefulness of proliferative activity and tumor suppressor gene activity as predictors of tumor growth has been shown [13,18,38–40,44], but the relationship between the DT and proliferative activity or tumor suppressor gene activity has never been clarified. Malaise et al. [46] have collected data on the labeling index of human solid tumors from the literature and have pooled them with their own results, comparing the labeling index with previously reported DTs. To our knowledge, this was one of the two reports that compared the kinetics of cell proliferation with the DT. In this study, however, the kinetics of cell proliferation and the DTs were not derived from the same tumors. In contrast, the results of our study are based on the relationship between the proliferative activity and the growth rate of the same tumors. In the only other report, Ogura et al. [21] showed a correlation between the DT and the activity of AgNOR (a parameter of proliferative activity) in lung adenocarcinoma. Based on the work of Ogura et al. [21], we expected that the DT would also correlate with proliferative activity in colorectal carcinomas, assuming that the growth process of colorectal carcinoma is identical to that of lung carcinoma. In our study, however, no relationship was seen for colorectal carcinomas. This suggests that the growth pattern of colorectal carcinoma is not identical to that of lung carcinoma. One difference between these tumors may be that the degree of cell loss is lower in lung tumors than in colorectal carcinomas [47]. Terz et al. [47] analyzed the cell cycles of seven

cases of human solid tumors (lung, maxillary antrum, malignant Schwannoma, malignant melanoma, colon carcinoma, and two breast carcinomas). They have reported a significant discrepancy between the calculated DT and the measured DT, and calculated the cell loss on the basis of this difference. The reported cell loss in the colon carcinoma was between 36% and 49%. Cell loss may influence the growth rate. On the basis of the work by Collins et al. [1,2], we calculated the DT under the hypothesis that the growth rate of a tumor is constant if the environments of tumor growth are constant. In lung carcinomas, the environments of tumor growth are thought to be constant [48]. However, in colorectal carcinomas, the environments of tumor growth may change as the tumor stage changes [48]. In our study, an accelerated growth pattern, especially in late stages, was shown in cases with more than two follow-up BEs. This suggests that the growth rate of colorectal carcinoma is not constant. Fujita et al. [48] have reported a similar growth pattern with a greater rate of longitudinal growth during the first, compared to the second, observation period in gastric carcinomas. They suggested that the marked difference in growth rates was due to a difference in cell loss rates in the two situations. Superficial spreading carcinoma loses a large proportion of carcinoma cells by desquamation, digestion, and mechanical friction on the luminal surface. In contrast, deep tumors lose carcinoma cells only to the extent that the growth of the stroma or of an efficient supply of nutrients and oxygen does not catch up with the potential growth of the tumor. This suggests that the environments of tumor growth are not identical during the life of gastrointestinal carcinomas. Tumors that invade deep portions of the colorectal wall may lose fewer tumor cells than those that invade superficial portions, thus the growth rate of tumors accelerates during later stages. As deep tumors are of a more advanced stage, they have more prominent intratumoral heterogeneity.

We conclude that the exponential growth of human colorectal tumors is modified by a number of factors, such as tumor heterogeneity and the loss of cells by surface desquamation. It is not possible to predict the growth rate of colorectal carcinomas by measuring tumor proliferative activity or tumor suppressor gene activity.

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